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COMMONWEALTH OF AUSTRALIA

IN THE MATTER OF: Australian Patent
Application 696764 (73941/94). In the name of:
Human Genome Sciences Inc.

-and-

IN THE MATTER OF: Opposition thereto by
Ludwig Institute for Cancer Research, under
Section 59 of the Patents Act.

STATUTORY DECLARATION

I, Susan Power of Cell & Molecular Technologies, Inc., Phillipsburg, New Jersey, United
States of America, declare as follows:

1. At the request of the Patent Attorneys representing Human Genome Sciences ("HGS") in connection with the Ludwig Institute for Cancer Research Opposition to the issuance of HGS Australian Patent Application 696764, in the name of HGS, entitled: "Vascular Endothelial Growth Factor-2" ("the HGS patent specification"), I performed certain experiments as described in a Statutory Declaration executed December 13, 2000 ("Power Declaration I"). The Patent Attorneys representing HGS have now requested that I provide additional information regarding those experiments and carry out additional experiments.
2. In particular, I have been asked to clarify the construction of the expression vectors described in Power Declaration I used to determine whether the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells.
3. The Patent Attorneys representing HGS have requested that I perform additional experiments to determine whether the VEGF-2 coding sequence contained in ATCC Deposit No. 75698 fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein

from eukaryotic cells. Further, the Patent Attorneys representing HGS have requested that I construct an expression vector containing the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence using only the VEGF-2 coding sequence contained in the ATCC Deposit No. 75698 and the nucleotide sequence of Figure 1 of the HGS application which contains a nucleotide sequence encoding the 350 amino acid form of VEGF-2, and methods and materials known as of March, 1994. I have done this and the experiments I have conducted are described herein.

The Design and Construction of the Expression Vectors Used in the Experiments Described in Power Declaration I

4. The Patent Attorneys representing HGS had previously asked that I perform experiments in order to determine whether the 350 amino acid form of VEGF-2 would be secreted from cells when attached to a heterologous signal sequence. To achieve this aim, I transfected eukaryotic cells with expression vectors encoding either (1) the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, or (2) the 419 amino acid form of VEGF-2. The transfected cells were grown and allowed to express the gene products encoded by the vectors. At various time points, both the cell lysates and culture medium were assayed for the presence of VEGF-2 protein. The presence of VEGF-2 protein in either the cell lysates or culture medium was determined by Western Blot analysis of the samples. I have reviewed my notebooks documenting the experiments I performed to achieve the aims of the experiments described in Power Declaration I and provide the following details:
5. For the experiments in Power Declaration I, I was asked to obtain the VEGF-2 DNA directly from the American Tissue Culture Collection (ATCC). I did not obtain any constructs from HGS. The only VEGF-2 clones I obtained were ATCC Deposit No. 97149 and ATCC Deposit No. 75698. The Patent

Attorneys representing HGS provided to me Figure 1 of the HGS patent specification which contains a nucleotide sequence of the 350 amino acid form of VEGF-2. The HGS Patent Attorneys also provided to me the nucleotide sequence of the 419 amino acid form of VEGF-2. It was my understanding that a nucleotide sequence encoding the 350 amino acid form of VEGF-2 was contained in ATCC Deposit No. 75698 and the nucleotide sequence encoding the 419 amino acid form of VEGF-2 was contained in ATCC Deposit No. 97149. It was also my understanding that the amino acid sequence of the 350 amino acid form of VEGF-2 corresponds to residues 70 to 419 of the 419 amino acid form of VEGF-2.

6. As I was under significant time constraints to complete the experiments, I elected to generate the DNA for the expression constructs using only the clone contained in ATCC Deposit No. 97149. Because I was using ATCC Deposit No. 97149 to generate the DNA, I also consulted the nucleotide sequence information relating to the 419 amino acid form of VEGF-2. I considered this to be a reasonable approach since the coding sequences for both the 419 and 350 amino acid forms of VEGF-2 are contained in ATCC Deposit No. 97149. Thus, I isolated the nucleotide sequences encoding the 419 amino acid form of VEGF-2 as well as the 350 amino acid form of VEGF-2 using ATCC Deposit No. 97149 as the sole source of VEGF-2 coding sequences.
7. My understanding of the goals of the experiments described in Power Declaration I was to demonstrate that the 350 amino acid form of VEGF-2 could be successfully expressed and secreted when expressed as taught by the HGS patent specification, *i.e.*, using a heterologous signal sequence. I did not inform the patent attorneys representing HGS at the time of carrying out these experiments nor at the time of signing Power Declaration I that I had isolated the 350 amino acid form of VEGF-2 from the ATCC Deposit No. 97149 clone. It was only when they asked on or about September 24, 2001 for

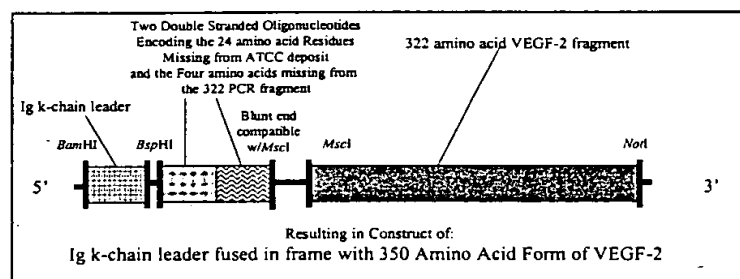
further clarification of the experiments that I conducted that I informed them of these details.

8. I have now been asked to redesign my experimental protocol to specifically use the VEGF-2 coding sequence contained in ATCC Deposit No. 75698 to generate the expression construct containing the 350 amino acid form of VEGF-2. I have been asked that I perform the experiments using the VEGF-2 coding sequence contained in ATCC Deposit No. 75698 to determine whether the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells.
9. I have provided the Patent Attorneys for HGS with the details of a sequence analysis of the VEGF-2 coding sequence contained in ATCC Deposit No. 75698. The VEGF-2 clone contained in the ATCC Deposit No. 75698 lacks 24 amino acids at the N-terminal end of the 350 amino acid form of VEGF-2, and corresponds to residues 94 to 419 of the 419 amino acid form of VEGF-2, *i.e.*, a 326 amino acid form of VEGF-2. I have also been asked to perform experiments to determine if the 326 amino acid form of VEGF-2 as encoded by a nucleotide sequence contained in ATCC Deposit No. 75698 fused to a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells.
10. Even though ATCC Deposit No. 75698 lacks the complete coding sequence for the 350 amino acid form of VEGF-2, a molecular biologist as of March 1994 would be able to recreate the 350 amino acid form of VEGF-2 given the description of the complete sequence in the HGS patent specification (as described below) and that is the course I could have taken at that time and I would have expected other molecular biologists to have been able to do the same. I generated an expression vector containing the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, using only the

ATCC Deposit No. 75698 and the sequence of Figure 1 in the HGS patent specification, and techniques and materials routinely known and used in the art as of March 1994.

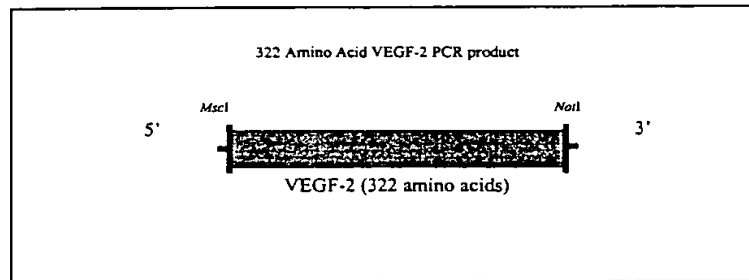
The Expression Vector Containing the 350 Amino Acid Form of VEGF-2 Is Generated Using Only ATCC Deposit No. 75698 and Figure 1 of the HGS Patent Specification

11. The general design of the expression vector containing the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence is as follows:

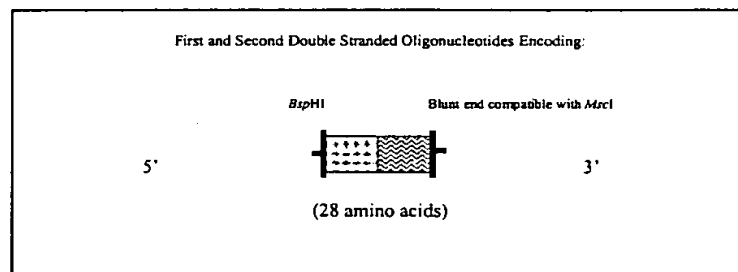


12. Since all that I had at my disposal were ATCC Deposit No. 75698 and Figure 1 of the HGS patent specification, I did the following:
- 12.1 First, I chose to directly isolate a nucleotide sequence encoding the C-terminal 322 residues of the 326 amino acid form of VEGF-2. The 322 residues corresponding to residues 98 to 419 of the 419 amino acid form of VEGF-2 were amplified by PCR from ATCC Deposit No. 75698. I chose to isolate a VEGF-2 fragment of 322 amino acids to facilitate the cloning of the VEGF-2 coding sequence in frame into the expression constructs. To do so, I designed primers based on the sequence provided in Figure 1 of the HGS patent specification, the sequence of ATCC Deposit No. 75698, and the sequence of restriction enzyme recognition sites, *e.g.*, *MscI* and *NotI*. The resulting 322 amino

acid fragment of VEGF-2 amplified from ATCC Deposit No. 75698 was digested with *Msc* I and *Not* I.

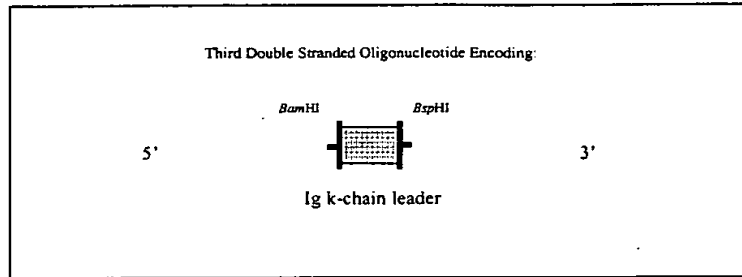


- 12.2 Using a nucleotide sequence encoding the 350 amino acid form of VEGF-2 contained in Figure 1 of the HGS specification, I designed two double stranded oligonucleotides to encode (once ligated together) a 28 amino acid VEGF-2 fragment. This fragment encompasses the 24 amino acids missing from ATCC Deposit No. 75698 and the additional 4 amino acids missing from the 322 amino acid fragment of the 326 form of VEGF-2. Specifically, once ligated together, the oligonucleotides were designed to result in the generation of a 28 amino acid fragment engineered to have a 5' end with a *Bsp*HI restriction site overhang and a 3' blunt end compatible with a *Msc* I restriction site as shown below. Methods and materials for generating such double stranded oligonucleotides were routine and known by March, 1994.



- 12.3 A third double stranded oligonucleotide encoding the secretion signal sequence of the Ig k-chain leader signal sequence that was also used in

the experiments described in Power Declaration I was engineered to contain a *Bam* HI restriction enzyme overhang at the 5' end and a *Bsp* HI restriction enzyme overhang at the 3' end as shown below. Ig k-chain leader signal sequence was a recognized signal sequence available as of March, 1994.



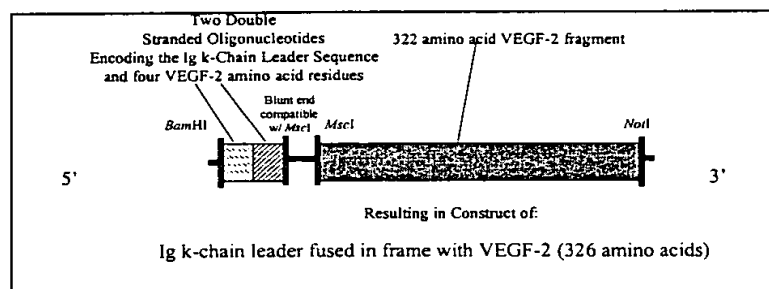
12.4 The 322 amino acid VEGF-2 fragment and the three double stranded oligonucleotides described above were ligated and subcloned at once into the *Not* I/*Bam* HI sites of the expression vector pCMV-I which was described in Power Declaration I. The resulting expression vector contains the construct as described in ¶ 11 above. The VEGF-2 sequence is under the control of a CMV-I promoter, a promoter routinely used as of March, 1994.

12.5 The resulting 350 amino acid form of VEGF-2 construct was sequenced and confirmed to be correct and is detailed in Appendix I.

13. The design of the expression vector containing the VEGF-2 coding sequence found in ATCC Deposit No. 75698 used in the study results in a construct with the 326 amino acid form of VEGF-2 linked to a heterologous sequence and is as follows:

14. To generate the construct, the 322 amino acid VEGF-2 fragment flanked with a *Msc* I site at the 5' end and the *Not* I site at the 3' end was generated as

described above (see ¶12.1). I designed two double stranded oligonucleotides that once ligated together encoded the Ig k-chain leader signal sequence and the four amino acid residues corresponding to residues 94 to 97 of the 419 amino acid form of VEGF-2, *i.e.*, the first four residues of the 326 amino acid form of VEGF-2 of ATCC Deposit No. 75698 engineered to contain a 3' blunt end compatible with a *MscI* restriction site and a 5' *Bam* HI site. The 322 amino acid VEGF-2 fragment was simultaneously fused in frame with the two double stranded oligonucleotides, as shown below, and subcloned into the expression vector pCMV-I *Bam* HI/ *Not* I sites. Again, the VEGF-2 sequence is under the control of a CMV-I promoter, a promoter routinely used as of March 1994.



15. The sequence of the resulting 326 amino acid form of VEGF-2 construct was confirmed to be correct and is detailed in Appendix II, attached hereto.
16. For purposes of the following experiments, I used the expression vector encoding the 419 amino acid form of VEGF-2 described in Power Declaration I (see Power Declaration I ¶¶ 3 to 6).
17. As set out in Power Declaration I, the sequence of the construct was confirmed to be correct and is detailed in Appendix III, attached hereto.

Using Only the VEGF-2 Clone Contained in ATCC Deposit No. 75698 Fused in Frame with a Heterologous Signal Sequence, Expression and Secretion of VEGF-2 Is Achieved

18. The Patent Attorneys for HGS requested that I perform the following experiments in order to determine whether using only the 350 amino acid form or the 326 amino acid form of VEGF-2 contained in ATCC Deposit No. 75698 fused in frame with a heterologous signal sequence would result in the expression and secretion of VEGF-2 from eukaryotic cells.
19. The overall experimental design is as follows: eukaryotic cells were transfected with expression vectors encoding the 419 amino acid form of VEGF-2, the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, or the 326 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence. The transfected cells were cultured for 24 or 48 hours to allow for expression of the gene products encoded by each vector. In order to determine whether the VEGF-2 gene product was being expressed and secreted, the cell lysates and culture medium were collected to assay for the presence of VEGF-2. The presence of VEGF-2 in either the cell lysates or culture medium was determined by Western Blot analysis of the samples using a rabbit polyclonal antibody to VEGF-2 that recognizes all forms and fragments of VEGF-2. The same antibody was used in Power Declaration I to assay the presence of VEGF-2 proteins.
20. The three VEGF-2 constructs encoding the 419, 350 and 326 amino acid forms of VEGF-2 each were transiently transfected in duplicate, using the lipofectin method into the Human Embryonic Kidney cell line, HEK-293 tsA-0. The method of transfection and the cell line were both routinely used as of March, 1994. As a control for transfection efficiency, each construct was co-transfected with the vector pCMV- β -gal. The efficiency of transfection was determined by β -gal staining 48 hours after transfection. As a negative control,

the vector pCMV-I without the addition of any VEGF-2 coding sequences was transfected in parallel.

21. The transfection design is as follows:

- 6 dishes transfected with: pCMV-I-VEGF-419;
- 6 dishes transfected with: pCMV-I-signal sequence-VEGF-350;
- 6 dishes transfected with: pCMV-I-signal sequence-VEGF-326;
- 6 dishes transfected: pCMV-I;
- 1 dish transfected with: pCMV-I-VEGF-419 + pCMV- β -gal;
- 1 dish transfected with: pCMV-I-signal sequence-VEGF-350 + pCMV- β -gal; and
- 1 dish transfected with: pCMV-I-signal sequence-VEGF-326 + pCMV- β -gal.

22. After transfection, DMEM medium containing 3% serum was added to the cells. Aliquots of cell extracts and conditioned medium were prepared from each transfection at: T₀ hours, T₂₄ hours and T₄₈ hours, in duplicate.

23. At the time of harvesting the cells and medium were treated as follows:

Medium: Harvested medium was concentrated 3 fold using Centricon 10 concentrator devices. One volume of 2 x PAGE loading dye was added to each sample.

Cell Extracts: The cells were harvested by trypsinization and collected by centrifugation. The cell pellet was resuspended and lysed, and one volume of 2 x PAGE loading dye was added to each sample.

24. To determine the transfection efficiency, dishes transfected with the pCMV- β -gal construct were fixed and stained for β -gal activity 48 hours after transfection. All dishes were found to have the same percentage of transfected cells (70%).

25. Each protein sample was subjected to Western analysis as outlined below. Prior to loading on to a 12% (w/v) Tris-Glycine SDS-Polyacrylamide gel, the samples were boiled for 5 minutes and cooled on ice. The two end lanes of each gel contained the appropriately sized molecular weight markers to estimate the migration rate of proteins predicted to run in the 16 to 85 kDa size range. The samples were electrophoresed according to standard conditions.
26. Following electrophoresis, the samples were transferred to a PVDF membrane. Each membrane was blocked by a one hour incubation in phosphate buffered saline (PBS) containing 3% Bovine Serum Albumin (BSA). The blot was then incubated at 4°C in PBS containing 0.1% BSA and 500ng/ml of purified rabbit anti-VEGF-2 antibody, a polyclonal antibody which recognizes all immunogenic fragments of VEGF-2. After three 5 minute washes in PBS containing 0.1% Tween, the blot was incubated for 1 hour in PBS containing 0.1% BSA and a 1:3000 dilution of Goat Anti-Rabbit IgG Horse Radish Peroxidase (HRP) conjugated antibody. The blot was washed six times for 5 minutes in PBS containing 0.1% BSA. The blot was developed with 2ml/blot of ECL detection reagent (obtained from Amersham) for one minute and then exposed directly to Polaroid films for approximately 2-3 seconds.
27. The result of the experiment is shown in Figure 1, attached hereto. The samples included in the figure are as follows:

Immunoblot analysis of VEGF constructs transiently expressed in HEK293T cells

Lane	Pellet/ Supernatant	Construct (419, 350, 326, or neg. control)	T (h) post-transfection
<i>Gel 1</i>			
1	P	Negative control	24
2	S	Negative control	24
3	P	350-signal	24
4	S	350-signal	24
5	P	326-signal	24

6	S	326-signal	24
7	P	419	48
8	S	419	48
Gel 2			
1	P	Negative control	24
2	S	Negative control	24
3	P	350-signal	24
4	S	350-signal	24
5	P	326-signal	24
6	S	326-signal	24
7	P	419	48
8	S	419	48
Gel 3			
1	P	Negative control	24
2	S	Negative control	24
3	P	419	24
4	S	419	24
5	P	419	24
6	S	419	24
7	P	350-signal	48
8	S	350-signal	48
9	P	419	48
10	S	419	48
Gel 4			
1	P	Negative control	48
2	S	Negative control	48
3	P	350-signal	48
4	S	350-signal	48
5	P	326-signal	48
6	S	326-signal	48
7	P	326-signal	48
8	S	326-signal	48

9	P	419	48
10	S	419	48

31. The Western Blot analysis indicates a broad band resolving at approximately 30kDa was present in the medium collected from the transfection of the 419 amino acid VEGF-2 construct, the 350 amino acid VEGF-2 signal sequence construct, and the 326 amino acid VEGF-2 signal sequence construct (see Figure 1, attached hereto as Appendix IV). The secreted protein was visible at 24 hours and 48 hours after transfection. The secreted product from cells containing the 419 amino acid VEGF-2 construct, the 350 amino acid VEGF2 signal sequence construct, and the 326 amino acid VEGF-2 signal sequence construct are all the same approximate size.

AND I declare that all the statements made in this Declaration are of my own are true in every particular, and that all statements made on information and belief are believed to be true.

Sworn by the said Susan Power, Susan Power at
Phillipsburg, New Jersey, on this 22nd day of March 2002;
before me Gean Rotmistrenko
Notary Public

GEAN ROTMISTRENKO
Notary Public, State of New York
No. 41-4778718
Qualified in Queens County
Certificate Filed in New York County
Commission Expires October 31, 2025

VEGF-350+Signal

	BamHI	NcoI																
				Met	Glu	Thr	Asp	Thr	Leu	Leu	Leu	Trp	Val	Leu	Leu	Leu		
1	GGATCCGCCA	CCATGGAGAC	AGACACACTC	CTGCTATGGG	TACTGCTGCT													
1	CCTAGGCGGT	GGTACCTCTG	TCTGTGTGAG	GACGATACCC	ATGACGACGA													
51	CTGGGTTCCA	GGTTCCACTG	GTGACATGAC	TGTACTCTAC	CCAGAATATT													
1	GACCCAAGGT	CCAAGGTGAC	CACTGTACTG	ACATGAGATG	GGTCTTATAA													
101	GGAAAATGTA	CAAGTGTGAG	CTAAGGAAAG	GAGGCTGGCA	ACATAACAGA													
1	CCTTTTACAT	GTTACAGTGC	GATTCCTTTC	CTCCGACCGT	TGTATTGTCT													
151	GAACAGGCCA	ACCTCAACTC	AAGGACAGAA	GAGACTATAA	AATTTGCTGC													
1	CTTGTCGGT	TGGAGTTGAG	TTCTGTCTT	CTCTGATATT	TTAAACGACG													
	BgIII																	
201	AGCACATTAT	AATACAGAGA	TCTTGAAAAG	TATTGATAAT	GAGTGGAGAA													
1	TCGTGTAATA	TTATGTCTCT	AGAACTTTTC	ATAACTATTA	CTCACCTCTT													
	SphI																	
251	AGACTCAATG	CATGCCACGG	GAGGTGTGTA	TAGATGTGGG	GAAGGAGTTT													
1	TCTGAGTTAC	GTACGGTGCC	CTCCACACAT	ATCTACACCC	CTTCCTCAAA													
	DraI AccI																	
301	GGAGTCGCGA	CAAACACCTT	CTTTAAACCT	CCATGTGTGT	CCGTCTACAG													
1	CCTCAGCGCT	GTTTGTGGAA	GAAATTTGGA	GGTACACACA	GGCAGATGTC													
351	ATGTGGGGGT	TGCTGCAATA	GTGAGGGGCT	GCAGTGCATG	AACACCAGCA													
1	TACACCCCCA	ACGACGTTAT	CACTCCCCGA	CGTCACGTAC	TTGTGGTCTG													
401	CGAGCTACCT	CAGCAAGACG	TTATTTGAAA	TTACAGTGCC	TCTCTCTCAA													
1	GCTCGATGGA	GTCGTTCTGC	AATAAACTTT	AATGTCACGG	AGAGAGAGTT													
451	GGCCCCAAAC	CAGTAACAAT	CAGTTTGGCC	AATCACACTT	CCTGCCGATG													
1	CCGGGGTTTG	GTCATTGTTA	GTCAAACGG	TTAGTGTGAA	GGACGGCTAC													
501	CATGTCTAAA	CTGGATGTTT	ACAGACAAGT	TCATTCCATT	ATTAGACGTT													
1	GTACAGATTT	GACCTACAAA	TGTCTGTTC	AGTAAGGTAA	TAATCTGCAA													
551	CCCTGCCAGC	AACACTACCA	CAGTGTGAGG	CAGCGAACAA	GACCTGCCCC													
1	GGGACGGTCG	TTGTGATGGT	GTCACAGTCC	GTCGCTTGTT	CTGGACGGGG													
601	ACCAATTACA	TGTGGAATAA	TCACATCTGC	AGATGCCTGG	CTCAGGAAGA													
1	TGGTTAATGT	ACACCTTATT	AGTGTAGACG	TCTACGGACC	GAGTCCTTCT													
651	TTTTATGTTT	TCCTCGGATG	CTGGAGATGA	CTCAACAGAT	GGATTCCATG													
1	AAAATACAAA	AGGAGCCTAC	GACCTCTACT	GAGTTGTETA	CCTAAGGTAC													

POWER DECLARATION II

APPENDIX I

VEGF-350+Signal

	Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val
701	ACATCTGTGG ACCAAACAAG GAGCTGGATG AAGAGACCTG TCAGTGTGTC TGTAGACACC TGGTTTGTTC CTCGACCTAC TTCTCTGGAC AGTCACACAG
	~~~~~ BsrBI
	Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp
751	TGCAGAGCGG GGCTTCGGCC TGCCAGCTGT GGACCCACACA AAGAACTAGA ACGTCTCGCC CCGAAGCCGG ACGGTCGACA CCTGGGGTGT TTCTTGATCT
	~~~~~
	Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys
801	CAGAAACTCA TGCCAGTGTG TCTGTAAAA CAAACTCTTC CCCAGCCAAT GTCTTTGAGT ACGGTCACAC AGACATTTTT GTTTGAGAAG GGGTCGGTTA
	~~~~~
	Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys
851	GTGGGGCCAA CCGAGAATTT GATGAAAACA CATGCCAGTG TGTATGTAAA CACCCCGGTT GGCTCTTAAA CTACTTTTGT GTACGGTCAC ACATACATTT
	~~~~~
	Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu
901	AGAACCTGCC CCAGAAATCA ACCCCTAAAT CCTGGAAAAT GTGCCTGTGA TCTTGGACGG GGTCTTTAGT TGGGGATTTA GGACCTTTTA CACGGACACT
	~~~~~
	Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His
951	ATGTACAGAA AGTCCACAGA AATGCTTGTT AAAAGGAAAG AAGTTCCACC TACATGCTTT TCAGGTGTCT TTACGAACAA TTTTCCTTTC TTCAAGGTGG
	~~~~~
	His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala
1001	ACCAACATG CAGCTGTTAC AGACGGCCAT GTACGAACCG CCAGAAGGCT TGGTTTGTAC GTCGACAATG TCTGCCGGTA CATGCTTGGC GGTCTTCCGA
	~~~~~
	Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser
1051	TGTGAGCCAG GATTTTCATA TAGTGAAGAA GTGTGTCGTT GCGTCCCTTC ACACTCGGTC CTAAAAGTAT ATCACTTCTT CACACAGCAA CGCAGGGAAG
	~~~~~
	NotI ~~~~~ EagI ~~~~~
	Ser Tyr Trp Lys Arg Pro Gln Met Ser ---
1101	ATATTGAAA AGACCACAAA TGAGCTAAGC GGCCGCG TATAACCTTT TCTGGTGTIT ACTCGATTCT CCGGCGC

VEGF 326+Signal

	BamHI	NcoI	Mel	Glu	Thr	Asp	Thr	Leu	Leu	Leu	Trp	Val	Leu	Lau	Leu
-1															
1	GGATCCGCCA	CCATGGAGAC	AGACACACTC	CTGCTATGGG	TACTGTCTGT	CCTAGGCGGT	GGTACCCTCTG	TCTGTGTGAG	GACGATACCC	ATGACGACGA					
+1	Leu	Trp	Val	Pro	Gly	Ser	Thr	Gly	Asp	Arg	Glu	Gln	Ala	Asn	Leu Asn Ser Arg
51	CTGGGTTCCA	GGTTCCACTG	GTGACAGAGA	ACAGGCCAAC	CTCAACTCAA	GACCCAAGGT	CCAAGGTGAC	CACTGTCTCT	TGTCCGGTTG	GAGTTGAGTT					
														EgII	
+1	Arg	Thr	Glu	Glu	Thr	Ile	Lys	Phe	Ala	Ala	Ala	His	Tyr	Asn	Thr Glu Ile
101	GGACAGAAGA	GACTATAAAA	TTTGCTGCAG	CACATTATAA	TACAGAGATC	CCTGTCTTCT	CTGATATTTT	AAACGACGTC	GTGTAATATT	ATGTCTCTAG					
		EgIII										SphI			
+1	Leu	Lys	Ser	Ile	Asp	Asn	Glu	Trp	Arg	Lys	Thr	Gln	Cys	Met	Pro Arg Glu
151	TTGAAAAGTA	TTGATAATGA	GTGGAGAAAG	ACTCAATGCA	TGCCACGGGA	AACITTTTCA	AAC TATTACT	CACCTCTTTC	TGAGTTACGT	ACGGTGCCCT					Dra
+1	Glu	Val	Cys	Ile	Asp	Val	Gly	Lys	Glu	Phe	Gly	Val	Ala	Thr	Asn Thr Phe Phe
201	GGTGTGTATA	GATGTGGGGA	AGGAGTTTGG	AGTCGGGACA	AACACCTTCT	CCACACATAT	CTACACCCT	TCCTCAAACC	TCACGCGTGT	TTGTGGAAGA					
		DraI						AacI							
+1	Phe	Lys	Pro	Pro	Cys	Val	Ser	Val Tyr	Arg Cys	Gly Gly	Cys Cys	Asn Ser			
251	TTAAACCTCC	ATGTGTGTCC	GTCTACAGAT	GTGGGGGTTG	CTGCAATAGT	AATTTGGAGG	TACACACAGG	CAGATGCTA	CACCCCCAAC	GACGTTATCA					
+1	Glu	Gly	Leu	Gln	Cys	Met	Asn	Thr Ser Thr	Ser Tyr Leu	Ser Lys Thr Leu					
301	GAGGGGCTGC	AGTGCATGAA	CACCAGCACG	AGCTACCTCA	GCAAGACGTT	CTCCCCGACG	TCACGTACTT	GIGGTCGTGC	TCGATGGAGT	CGTTCTGCAA					
+1	Leu	Phe	Glu	Ile	Thr	Val	Pro	Leu Ser Gln	Gly Pro Lys	Pro Val Thr Ile Ser					
351	ATTTGAAATT	ACAGTGCCCTC	TCTCTCAAGG	CCCCAAACCA	GTAACAATCA	TAAACTTTAA	TGTCACGGAG	AGAGAGTTCC	GGGGTTTTGGT	CATTGTTAGT					
+1	Ser	Phe	Ala	Asn	His	Thr	Ser	Cys Arg Cys	Met Ser Lys	Leu Asp Val Tyr					
401	GTTTTGCCAA	TCACACTTCC	TGCCGATGCA	TGTCTAAACT	GGATGTTTAC	CAAACGGTT	AGTGTGAAGG	ACGGCTACGT	ACAGATTTGA	CCTACAAATG					
+1	Arg	Gln	Val	His	Ser Ile	Ile	Arg Arg	Ser Leu Pro Ala	Thr Leu Pro Gln						
451	AGACAAGTTC	ATTCCATTAT	TAGACGTTCC	CTGCCAGCAA	CACTACCACA	TCTGTTCAAG	TAAGGTAATA	ATCTGCAAGG	GACGGTCGTT	GTGATGGTGT					
+1	Glr	Cys	Gln	Ala	Ala Asn Lys	Thr Cys Pro	Thr Asn Tyr Met	Trp Asn Asn His							
501	GTGTCAGGCA	GCGAACAAAG	CCTGCCCCAC	CAATTACATG	TGGAATAATC	CACAGTCCGT	CGCTTGTCT	GGACGGGGTG	GTTAATGTAC	ACCTTATTAG					
+1	His	Ile	Cys	Arg	Cys Leu Ala	Gln Glu Asp	Phe Met Phe	Ser Ser Asp Ala							
551	ACATCTGCAG	ATGCCTGGCT	CAGGAAGATT	TTATGTTTTT	CTCGGATGCT	TGTAGACGTC	TACGGACCGA	GTCCTTCTAA	AATACAAAAG	GAGCCTACGA					
+1	Gly	Asp	Asp	Ser	Thr Asp Gly	Phe His Asp	Ile Cys Gly	Pro Asn Lys Glu							
601	GGAGATGACT	CAACAGATGG	ATTCUATGAC	ATCTGTGGAC	CAAACAAGGA	CCTCTACTGA	GTTGTCTACC	TAAGGTACTG	TAGACACCTG	GTTTGTTECT					
											BsrBI				
+1	Glt	Leu	Asp	Glu	Glu Thr Cys	Gln Cys Val Cys	Arg Ala Gly	Leu Arg Pro Ala							
651	GCTEGATGAA	GAGACCTGTC	AGTGTGTCTG	CAGAGCGGGG	CTTCGGCCTG	CGACCTACTT	CTCTGGACAG	TCACACAGAC	GTCTCGCCCC	GAAGCCGGAC					

POWER DECLARATION II

APPENDIX II

VEGF 326+Signal

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+1 Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val
701 CCAGCTGTGG ACCCCACAAA GAACTAGACA GAAACTCATG CCAGTGTGTC
GGTCGACACC TGGGGTGTTC CTTGATCTGT CTTTGAGTAC GGTCACACAG
+1 Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp
751 TGAAAAACA AACTCTTCCC CAGCCAATGT GGGGCCAACC GAGAATTGTA
ACATTTTGTG TTGAGAAGGG GTCGGTTACA CCCCAGTTGG CTCTTAAAC
+1 Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
801 TGAAACACA TGCCAGTGTG TATGTAAAAG AACCTGCCCC AGAAATCAAC
ACTTTTGTGT ACGGTCACAC ATACATTTTC TTGGACGGGG TCTTTAGTTG
+1 Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
851 CCCTAAATCC TGGAAAATGT GCCTGTGAAT GTACAGAAAG TCCACAGAAA
GGGATTTAGG ACCTTTTACA CGGACACTTA CATGTCTTTC AGGTGTCTTT
+1 Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg
901 TGCTTGTTAA AAGGAAAGAA GTTCCACCAC CAAACATGCA GCTGTTACAG
ACGAACAATT TTCCTTTCTT CAAGGTGGTG GTTTGTACGT CGACAATGTC
+1 Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser
951 ACGGCCATGT ACGAACCGCC AGAAGGCTTG TGAGCCAGGA TTTTCATATA
TGCCGGTACA TGCTTGGCGG TCTTCCGAAC ACTCGGTCCT AAAAGTATAT
+1 Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met
1001 GTGAAGAGT GTGTCGTTGT GTCCCTTCAT ATTGGAAAAG ACCACAAATG
CACTTCTTCA CACAGCAACA CAGGGAAGTA TAACCTTTTC TGGTGTTTAC
+1 Ser
1051 AGCTAAGCGG CCGCG
TCGATTCGCC GGCGC

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419-VEGF-2

		EcoRI															
		Met His Leu Leu Gly Phe Phe Ser Val Ala															
1		GAATTCGTGG	GTCCTTCCAC	CATGCACTTG	CTGGGCTTCT	TCTCTGTGGC	CTTAAGCACC	CAGGAAGGTG	GTACGTGAAC	GACCCGAAGA	AGAGACACCG						
		SmaI															
		XmaI															
		AvaI															
		NarI															
51		GTGTTCTCTG	CTCGCCGCTG	CGCTGCTCCC	GGGTCTCTGC	GAGGCGCCCCG	CACAAGAGAC	GAGCGGCGAC	GCGACGAGGG	CCCAGGAGCG	CTCCGCGGGC						
101		CCGCGGCGCG	CGCCTTCGAG	TCCGGACTCG	ACCTCTCGGA	CGCGGAGCCC	GGCGGCGGGC	GCGGAAGCTC	AGGCCTGAGC	TGGAGAGCCT	GCGCCTCGGG						
151		GACGCGGGCG	AGGCCACGGC	TTATGCAAGC	AAAGATCTGG	AGGAGCAGTT	CTGCGCCCGC	TCCGGTGCCG	AATACGTTCT	TTTCTAGACC	TCCTCGTCAA						
		BspHI															
201		ACGGTCTGTG	TCCAGTGTAG	ATGAATCAT	GACTGTACTC	TACCCAGAAT	TGCCAGACAC	AGGTCACATC	TACTTGAGTA	CTGACATGAG	ATGGGTCTTA						
251		ATTGGAAAAT	GTACAAGTGT	CAGCTAAGGA	AAGGAGGCTG	GCAACATAAC	TAACCTTTTA	CATGTTTACA	GTCGATTCCT	TTCTCTCGAC	CGTTGTATTG						
301		AGAGAACAGG	CCAACCTCAA	CTCAAGGACA	GAAGAGACTA	TAAAATTTGC	TCTCTTGTC	GGTTGGAGTT	GAGTTCTTGT	CTTCTCTGAT	ATTTTAAACG						
351		TGCAGCACAT	TATAATACAG	AGATCTTGAA	AAGTATTGAT	AATGAGTGGA	ACGTCGTGTA	ATATTATGTC	TCTAGAATT	TTTATAACTA	TTACTCACCT						
		SphI															
401		GAAAGACTCA	ATGCATGCCA	CGGGAGGTGT	GTATAGATGT	GGGGAAGGAG	CTTCTGAGT	TACGTACGGT	GCCCTCCACA	CATATCTACA	CCCTTCCTC						
		DraI															
		AccI															
450		TTTGGAGTCG	CGACAAACAC	CTTCTTTAAA	CTCCATGTG	TGTCCGTCTA	AAACCTCAGC	GCTGTTTGTG	GAAGAAATTT	GGAGGTACAC	ACAGGCAGAT						
		AccI															
501		CAGATGTGGG	GGTTGCTGCA	ATAGTGAGGG	GCTGCAGTGC	ATGAACACCA	GTCTACACCC	CCAACGACGT	TATCACTCCC	CGACGTCACG	TACTTGTGGT						
551		GCACGAGCTA	CCTCAGCAAG	ACGTTATTTG	AAATTACAGT	GCCTCTCTCT	CGTGCTCGAT	GGAGTCGTTT	TGCAATAAAC	TTTAATGTCA	CGGAGAGAGA						
601		CAAGGCCCCA	AACCAAGTAAC	AATCAGTTTT	GCCAATCACA	CTTCCTGCGG	GTTCCGGGGT	TTGGTCATTG	TTAGTCAAAA	CGGTTAGTGT	GAAGGACGGC						

POWER DECLARATION II

APPENDIX III

419-VEGF-2

+1 Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg
 651 ATGCATGTCT AAACCTGGATG TTTACAGACA AGTTCATTCC ATTATTAGAC
 TACGTACAGA TTTGACCTAC AAATGTCTGT TCAAGTAAGG TAATAATCTG
 +1 Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys
 701 GTTCCTGCC AGCAACACTA CCACAGTGTG AGGCAGCGAA CAAGACCTGC
 CAAGGGACGG TCGTTGTGAT GGTGTCACAG TCCGTCGCTT GTTCTGGACG
 +1 Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu
 751 CCCACCAATT ACATGTGGAA TAATCACATC TGCAGATGCC TGGCTCAGGA
 GGGTGGTTAA TGTACACCTT ATTAGTGTAG ACGTCTACGG ACCGAGTCCT
 +1 Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His
 801 AGATTTTATG TTTTCCTCGG ATGCTGGAGA TGACTCAACA GATGGATTCC
 TCTAAAATAC AAAAGGAGCC TACGACCTCT ACTGAGTTGT CTACCTAAGG
 +1 His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys
 851 ATGACATCTG TGGACCAAAC AAGGAGCTGG ATGAAGAGAC CTGTCAGTGT
 TACTGTAGAC ACCTGGTTTG TTCCTCGACC TACTTCTCTG GACAGTCACA
 BsrBI
 +1 Val Cys Arg Ala Gly Leu Arg Pro Asn Ser Cys Gly Pro His Lys Glu Leu
 901 GTCTGCAGAG CGGGGCTTCG GCCTGCCAGC TGTGGACCCC ACAAGAAGCT
 CAGACGTCTC GCCCCGAAGC CGGACGGTCG ACACCTGGGG TGTTCCTTGA
 +1 Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln
 951 AGACAGAAAC TCATGCCAGT GTGTCTGTAA AAACAAACTC TTCCCCAGCC
 TCTGTCTTTG AGTACGGTCA CACAGACATT TTTGTTTGAG AAGGGGTCGG
 +1 Glu Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys
 1001 AATGTGGGGC CAACCGAGAA TTTGATGAAA ACACATGCCA GTGTGTATGT
 TTACACCCCG GTTGGCTCTT AAACACTTTT TGTGTACGGT CACACATACA
 +1 Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys
 1051 AAAAGAACCT GCCCCAGAAA TCAACCCCTA AATCCTGGAA AATGTGCCTG
 TTTCTTGGG CGGGGTCTTT AGTTGGGGAT TTAGGACCTT TTACACGGAC
 +1 Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His
 1101 TGAATGTACA GAAAGTCCAC AGAATGCTT GTTAAAAGGA AAGAAGTTCC
 ACTTACATGT CTTTCAGGTG TCTTTACGAA CAATTTTCCT TTCTTCAAGG
 +1 His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys
 1151 ACCACCAAAC ATGCAGCTGT TACAGACGGC CATGTACGAA CCGCCAGAAG
 TGGTGGTTTG TACGTCGACA ATGCTGCCG GTACATGCTT GCGGGTCTTC
 +1 Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro
 1201 GCTTGTGAGC CAGGATTTTC ATATAGTGAA GAAGTGTGTC GTTGTGTCCC
 CGAACACTCG GTCCTAAAG TATATCACTT CTTACACAG CAACACAGGG
 NotI
 EagI
 +1 Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser ***
 1251 TTCATATTGG AAAAGACCAC AATGAGCTA AGCGGCCGCG
 AAGTATAACC TTTTCTGGTG TTTACTCGAT TCGCCGGCGC

Figure 1

